PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

In its capacity as elected Office

Date of mailing (day/month/year) 16 October 2000 (16.10.00)	
International application No. PCT/GB00/00414	**Applicant's or agent's file reference** SCB/50929/002
International filing date (day/month/year) 09 February 2000 (09.02.00)	**Priority date (day/month/year)** 09 February 1999 (09.02.99)
Applicant KNIGHT, Julian, Charles et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

08 September 2000 (08.09.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Zakaria EL KHODARY
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/00414

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 C07K14/47 A61K35/00 C12N5/10 C12N15/85

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BRINKMAN B M ET AL: "Relevance of the tumor necrosis factor alpha (TNF alpha) -308 promoter polymorphism in TNF alpha gene regulation 'see comments!." JOURNAL OF INFLAMMATION, (1995-96) 46 (1) 32-41. , XP000907438 the whole document	33-36
X	WO 97 42820 A (UNIV DUKE) 20 November 1997 (1997-11-20) the whole document	22
A	UDALOVA I A ET AL: "Complex NF-kappaB interactions at the distal tumor necrosis factor promoter region in human monocytes." JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 AUG 14) 273 (33) 21178-86. , XP002139037	1-36

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 May 2000	15/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3018	Reuter, U

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte. onal Application No
PCT/GB 00/00414

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	DROUET, C. ET AL: "Enhancers and transcription factors controlling the inducibility of the tumor necrosis factor-.alpha. promoter in primary macrophages" J. IMMUNOL. (1991), 147(5), 1694-700 , XP002139038	1-36
A	KAIJZEL E L ET AL: "Functional analysis of a human tumor necrosis factor alpha (TNF -alpha) promoter polymorphism related to joint damage in rheumatoid arthritis." MOLECULAR MEDICINE, (1998 NOV) 4 (11) 724-33. , XP000907439 the whole document	1-36
A	WILSON A G ET AL: "Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1997 APR 1) 94 (7) 3195-9. , XP002139040 the whole document	1-36
A	TAKASHIBA S ET AL: "Cloning and characterization of the human TNF alpha promoter region" GENE,NL,ELSEVIER BIOMEDICAL PRESS. AMSTERDAM, vol. 131, no. 131, 1993, pages 307-308-308, XP002103473 ISSN: 0378-1119 the whole document	1-36
A	WO 97 39146 A (CEDARS SINAI MEDICAL CENTER) 23 October 1997 (1997-10-23) the whole document	33-36
P,X	KNIGHT J C ET AL: "A polymorphism that affects OCT-1 binding to the TNF promoter region is associated with severe malaria 'see comments!." NATURE GENETICS, (1999 JUN) 22 (2) 145-50. , XP002139041 the whole document	1-36

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 00/00414

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9742820 A	20-11-1997	AU 3130597 A	05-12-1997
WO 9739146 A	23-10-1997	AU 2456197 A	07-11-1997
		AU 2725697 A	07-11-1997
		WO 9739147 A	23-10-1997

PATENT COOPERATION TREA[illegible]

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
SCB/50929/002		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 00/ 00414	09/02/2000	09/02/1999
Applicant		
ISIS INNOVATION LTD. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

   [X] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 2

   [ ] as suggested by the applicant.

   [X] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
SCB/50929/002		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/00414	09/02/2000	09/02/1999
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant ISIS INNOVATION LTD. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 13 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 10 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08/09/2000	20.04.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Favre, N Telephone No. +49 89 2399 7363

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/00414

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-34 as originally filed

Claims, No.:

1-41 with telefax of 28/02/2001

Drawings, sheets:

1/7-7/7 as originally filed

Sequence listing part of the description, pages:

1-2, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 22.

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 22 are so unclear that no meaningful opinion could be formed (*specify*):
   see separate sheet

   ☒ the claims, or said claims Nos. 22 are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-21, 23-41
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-3, 7-9, 23-27, 30-32, 36-41
	No:	Claims	4-6, 10-21, 28, 29, 33-35
Industrial applicability (IA)	Yes:	Claims	1-21 and 23-41
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claim 22 refers to a compound identified by the methods of claims 15-21. In the application as filed, there is however no example of any such compound (Rule 5.1 a)(v) PCT). Moreover, such a compound is not a product **directly produced** by the screening methods of claims 15-21, which relate to methods of identification. It is rather a product that has been **identified** using said methods.
Therefore, claim 22 is not considered to be supported by the description in the sense of Article 6 PCT.

Moreover, the subject-matter of claim 22 is also not considered to be sufficiently disclosed in the application as filed for the skilled person to carry them out and thus do not meet the requirements of Article 5 PCT as there is no indication whatsoever in the application as filed of a compound falling within the scope of this claim.

According to the above objections, it is not possible to assess claim 22 for novelty and inventive step in the sense of Articles 33(2) and 33(3) PCT.

Re Item IV

Lack of unity of invention

The application as filed lacks unity within the meaning of Rule 13.1 PCT. However, it was chosen, according to Rule 68.1 PCT, not to invite the applicant to restrict or to pay additional fees.

The following groups of invention were found in the present application:

I) **Claims 1-3, 7-9, 12-16 (partially), 17,18, 20 (partially), 21 (partially), 23-27, 33-35, 36 (partially), 37, 38, 40 (partially) and 41**: These claims define DNA binding proteins, nucleic acid molecules and methods, all of which relate to the α site (-404 - -374) of the *TNFα* promoter, said α site comprising a phenotypically relevant A to G transition polymorphism at position -376.

II) **Claims 4-6, 10, 11, 12-16 (partially), 19, 20 (partially), 21 (partially), 28-32, 36 (partially), 39 and 40 (partially)**: These claims define DNA binding proteins, nucleic acid molecules and methods, all of which relate to the β site (-371 - -352) of the *TNFα* promoter, said β site beginning 3 nucleotides downstream of the α site.

Apart from the fact that the α and β sites are immediately adjacent, there is no evidence, neither in the present application nor in the prior art, that both sites are functionally linked, for instance for the modulation of the TNFα gene expression. Therefore, the above two groups of inventions are not so linked by a special technical feature as to form a single general inventive concept (Rule 13.1 PCT).

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statem nt

1. Document D1 (Gene, 1993, **131**:307-308) discloses the nucleic acid sequence of the *TNFα* promoter region. In Figure 1, D1 discloses that the hepatocyte-specific nuclear protein-1 (H-APF-1) specifically binds to a DNA sequence corresponding to SEQ ID NO: 1.
Moreover, it is known in the art, cf. document D2 (Nature Genetics, 1999, **22**:145-150) referring to a publication of Fletcher *et al.* in Cell, 1987, that OCT-1, another DNA binding protein, specifically binds to a DNA sequence corresponding to SEQ ID NO: 2.
However, none of the prior art documents at hand discloses a DNA binding protein capable of binding specifically both to a DNA having the sequence of SEQ ID NO:1 and to a DNA having the sequence of SEQ ID NO:2. The subject-matter of claim 1 is thus novel.
Furthermore, it is shown in the description that the binding of the claimed DNA binding protein is modulated by the known -376A/G mutation and by the presence of OCT-1 (page 9, lines 1-18) and it is shown that this mutation is associated with a modulation of the TNFα gene expression (e.g. Example 5). Hence, it is strongly suggested that the claimed DNA binding protein plays a role in the modulation of the TNFα gene expression. Since none of the prior art documents at hand fairly suggest that a DNA binding protein similar to that defined in claim 1 has a similar function, the subject-matter of claim 1 is also considered to be inventive.
Therefore, the subject-matter of independent claim 1 meets the requirements of Articles 33(2) and 33(3) PCT.

1.1 Dependent claims 2 and 3 further define the protein of claim 1, which is considered to be novel and inventive. Dependent claims 2 and 3 thus also meet the requirements of Articles 33(2) and 33(3) PCT.

2. There is no indication in the prior art documents known to the International Preliminary Examination Authority of a protein specifically binding DNA having a sequence corresponding to SEQ ID NO: 3. The subject-matter of independent

claim 4 is thus novel in the sense of Article 33(2) PCT.
However, the mere fact that a protein binds to a particular DNA, without any further technical effect, e.g. modulation of the TNFα expression, is not considered to be a solution to a technical problem. Hence, no technical problem appears to be solved by the protein defined in claim 4.
Therefore, the subject-matter of independent claim 4 and dependent claims 5 and 6, which further define said protein using non-characterising features (see also Item VIII), is not considered to be an invention, and thus is not considered to be inventive in the sense of Article 33(3) PCT.

3. Document D1 discloses the complete sequence of the *TNFα* promoter region (Fig. 1). In other words, the nucleic acid of D1 comprises the sequence illustrated in SEQ ID NO: 1.
Document D3 (Journal of Inflammation, 1996, **46**:32-41) discloses that the -376 G to A transition polymorphism of the *TNFα* promoter region is known in the art.
However, the selection of a nucleic acid molecules restricted to the sequence of SEQ ID NO: 1 or of SEQ ID NO: 2 provides nucleic acid molecules containing binding sites for the novel and inventive DNA binding protein of claim 1, and as such are useful for evaluating the binding of said protein.
Therefore, the subject-matter of claims 7-9 is considered to be a novel and inventive selection, and thus fulfils the requirements of Articles 33(2) and 33(3) PCT.

3.1 However, the complete sequence of the *TNFα* promoter region disclosed in D1 (Fig. 1) also comprises the sequence illustrated in SEQ ID NO: 3.
In the light of the argumentation of point 2. above, the selection of a nucleic acid molecule restricted to the sequence of SEQ ID NO: 3 cannot be considered to be inventive. Therefore, the subject-matter of claims 10 and 11 is not considered to meet the requirements of Article 33(3) PCT.

4. Document D3, which is considered to represent the most relevant state of the art for the assessment of inventive step of claims 12-14, discloses (cf. page 34, column 1, line 3 - page 35, column 2, line 35) a reporter gene expression

construct comprising the α and β sites of the *TNFα* promoter region from which the subject-matter of claim 12 differs in that nucleic acid sequences of the *TNFα* promoter region other than sequences corresponding to SEQ ID NO: 1, 2, or 3 have to be absent.

4.1 The problem to be solved by independent claim 12 may therefore be regarded as providing a reporter gene expression construct for the study of a polymorphic site of the *TNFα* promoter region recognised by a DNA binding protein.

4.2 In view of the argumentation of point 1. above, the solution of said technical problem comprising the selection of SEQ ID NO:1 and 2 would meet the requirements of the PCT. However, insofar that the claimed solution includes SEQ ID NO:3 and in view of the argumentation of point 2. above, the subject-matter of claim 12 and of claim 13, which further define said construct with a feature which is standard for expression constructs, is not considered to be inventive in the sense of Article 33(3) PCT.

4.3 Accordingly, a cell line as defined in claim 14 also does not fulfill the requirements of Article 33(3) PCT.

5. Document D3, which is also considered to represent the most relevant state of the art for the assessment of inventive step of claims 15-21, discloses (cf. page 34, column 1, line 3 - page 35, column 2, line 35) a method from which the subject-matter of claim 15 differs in that another reporter gene expression construct is used, i.e. a construct which does not comprise nucleic acid sequences of the *TNFα* promoter region other than sequences corresponding to SEQ ID NO: 1, 2, or 3.

5.1 However, the reporter gene expression construct used in Example 5 of the present application (page 26, line 30) comprises the **complete** *TNFα* promoter region (~1.2 kb), i.e. comprises other factors which directly play a role in the modulation of the TNFα gene expression.
There is no indication whatsoever, either in the present application or in the prior art, that a reporter gene expression construct as claimed would lead to the

identification of a compound capable of modulating TNFα gene expression. Moreover, there is no indication whatsoever, either in the present application or in the prior art, that the nucleic acid sequence corresponding to SEQ ID NO: 3 plays any role in the modulation of the TNFα gene expression.

5.2 Modulation of the TNFα gene expression is the sole reason for the alleged inventiveness of these methods. The fact that the examples contained in the description show that the -376 polymorphism **in combination with** the rest of the **complete** *TNFα* promoter region plays a role in the modulation of the TNFα gene expression cannot not be regarded as sufficient evidence to lead to the inference that substantially all the claimed compounds possess this activity.
The requirements of Article 33(3) PCT are therefore not met by independent claim 15.

5.3 Similar objections also apply for the methods defined in claims 16-21 which thus do not meet the requirements of Article 33(3) PCT.

6. According to the objection of point 3. above, the nucleic acid molecules defined in claims 23 and 24 are considered to be novel and inventive in the sense of Articles 33(2) and 33(3) PCT.

6.1 However, according to the objection of point 3.1 above, the nucleic acid molecules defined in claims 28 and 29 are not considered to be inventive in the sense of Article 33(3) PCT.

7. Document D2, which is considered to represent the most relevant state of the art for the assessment of inventive step of claims 25-27 and 30-32 (see also Item II), refers to the nucleic acid molecules of claims 23 and 28.
There is however no suggestion in D2 or in any prior art documents known to the International Preliminary Examination Authority that would prompt the skilled person to immobilise said nucleic acid molecules to a solid support, for example in order to enrich a sample in specific DNA binding proteins (page 31-33 of the description).

Thus, claims 25-27 and 30-32 are considered to be novel and inventive in the sense of Articles 33(2) and 33(3) PCT.

8. Document D4 (Molecular Medicine, 1998, **4**:724-733), discloses (cf. abstract) a method of screening human individuals for predisposition to an inflammatory disease, which method comprises screening for the presence of a G to A transition polymorphism at position -376 in the promoter region of the *TNFα* gene. The subject-matter of independent claim 34 differs from this disclosure in that it is specified that the inflammatory disease is cerebral malaria.
However, the association between TNFα expression and cerebral malaria is well known in the art. Moreover, associations between G to A transition polymorphism in the *TNFα* promoter region and cerebral malaria are also known in the art, e.g. D3, page 40, column 1, lines 5-28. Therefore, using the method of D4 for screening human individuals for predisposition to cerebral malaria does not require an inventive activity from the skilled person. Claim 33 is thus not inventive in the sense of Article 33(3) PCT.

8.1 Finally, using PCR ELISA and oligonucleotide probes specific for each allele in a screening method require only routine selection from the person skilled in the art. Claims 34 and 35 are therefore not considered to be inventive in the sense of Article 33(3) PCT.

9. None of the prior art documents at hand discloses or fairly suggests methods of identifying compounds capable of disrupting a DNA/protein interaction occurring at the *TNFα* promoter, as defined in claims 36-41.
Said claims are thus considered to be novel and inventive in the sense of Articles 33(2) and 33(3) PCT.

Re Item VIII

Certain observations on the international application

1. Although claims 15 and 16 have been drafted as separate independent claims, they appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is sought. The aforementioned claims therefore lack conciseness. Moreover, lack of clarity of the claims as a whole arises, since the plurality of independent claims makes it difficult to determine the matter for which protection is sought, and places an undue burden on others seeking to establish the extent of the protection. Hence, claims 15 and 16 do not meet the requirements of Article 6 PCT.

1.1 Similarly, independent claims 17-19 also appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is sought.
Claims 17-19 do thus also not meet the requirements of Article 6 PCT.

1.2 A similar objection also applies for claims 37-39. Said claims do therefore not meet the requirements of Article 6 PCT.

2. The embodiment of the invention described on pages 26-28 (Example 5) does not fall within the scope of claims 12-21 (see also Item V, points 5-5.3). This inconsistency between the claims and the description leads to doubt concerning the matter for which protection is sought, thereby rendering the claims unclear (Article 6 PCT).

2.1 Moreover, according to the above objection, claims 12-21 are not experimentally supported by the description in the sense of Article 6 PCT.

3. Dependent claims 2 and 3 further define the protein of claim 1 by a particular molecular weight (between 16 and 26 kDa) which is not a feature which is unique for only one protein, or by the use of a particular process for the isolation of said protein. Both features do not characterise the claimed proteins, so that claims 2

and 3 are considered to be redundant and thus lack conciseness in the sense of Article 6 PCT.

3.1 A similar objection applies for claims 5 and 6, which thus do not meet the requirements of Article 6 PCT.

Claims:

1. A sequence-specific DNA binding protein which is capable of binding specifically to a DNA molecule having the sequence of nucleotides illustrated in SEQ ID NO: 1 or SEQ ID NO: 2.

2. A sequence-specific DNA binding protein as claimed in claim 1 having an electrophoretic mobility equivalent to a protein of molecular weight 21 +/- 5kDa when run on an SDS PAGE denaturing gel.

3. A sequence-specific DNA binding protein as claimed in claim 1 or claim 2 which is obtainable by

(a) preparing a crude nuclear extract from the human monocyte cell line U937;

(b) applying the nuclear extract of part (a) to a column of heparin sepharose CL-6B or phosphocellulose P11;

(c) applying a salt gradient from 100 to 2000mM to the column;

(d) collecting a fraction of the eluate which is enriched for the said sequence-specific DNA binding protein;;

(e) incubating the enriched material obtained in step (d) with double-stranded DNA affinity probes comprising the following sequence:

5'-GTTCTATCTTTTTCCTGCATCCTGTCTGGAAGTTA
CAAGATAGAAAAAGGACGTAGGACAGACCTTCAAT-5'

to allow the formation of complexes of the sequence-specific DNA binding protein bound to the DNA affinity probe; and

(f) recovering the sequence-specific binding protein from the complexes formed in step (e).

4. A sequence-specific DNA binding protein which is capable of binding specifically to a DNA molecule having the sequence of nucleotides illustrated in SEQ ID NO: 3.

5. A sequence-specific DNA binding protein as claimed in claim 4 having an electrophoretic mobility equivalent to a protein of molecular weight 30 +/- 5kDa when run on an SDS PAGE denaturing gel.

6. A sequence-specific DNA binding protein as claimed in claim 4 or claim 5 which is obtainable by:

(a) preparing a crude nuclear extract from the human monocyte cell line U937;

(b) applying the nuclear extract of part (a) to a column of heparin sepharose CL-6B or phosphocellulose P11;

(c) applying a salt gradient from 100 to 2000mM to the column;

(d) collecting the material eluted at 250-350mM salt for heparin sepharose or 500-600mM salt for P11 phosphocellulose;

(e) incubating the enriched material obtained in step (d) with double-stranded DNA affinity probes comprising the following sequence:

5'-TAGAAGGAAACAGACCACAGACCTG
ATCTTCCTTTGTCTGGTGTCTGGAC-5'

to allow the formation of complexes of the sequence-specific DNA binding protein bound to the DNA affinity probe; and

(f) recovering the sequence-specific binding protein from the complexes formed in step (e).

7. An isolated nucleic acid having the sequence of nucleotides illustrated in SEQ ID NO: 1.

8. An isolated nucleic acid having the sequence of nucleotides illustrated in SEQ ID NO: 2.

9. An isolated nucleic acid as claimed in claim 7 or claim 8 or a fragment thereof which is capable of specifically binding to the DNA binding protein of any one of claims 1 to 3.

10. An isolated nucleic acid having the sequence of nucleotides illustrated in SEQ ID NO: 3.

11. An isolated nucleic acid as claimed in claim 10 or a fragment thereof which is capable of specifically binding to the DNA binding protein of any one of claims 4 to 6.

12. A reporter gene expression construct comprising:

a reporter gene encoding a transcriptional and/or translational product which can be directly or indirectly detected; and

a transcriptional control element comprising one or more of the sequences of nucleotides illustrated in SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 in the substantial absence of any other nucleotide sequence from the TNF-α promoter, the transcriptional control element being operably linked to the reporter gene.

13. A reporter gene expression construct as claimed in claim 12 which further comprises one or more *cis*-acting promoter or enhancer elements from a heterologous promoter.

14. Cells transformed or transfected with the reporter gene expression construct of claim 12 or claim 13.

15. A method of identifying a compound capable of modulating TNF-α gene expression, which method comprises:

contacting a fragment of DNA comprising one or more of the sequences of nucleotides illustrated in SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 in the substantial absence of any other nucleotide sequences from a TNF-α promoter with a sample of the compound and detecting any specific binding of the compound to the fragment of DNA.

16. A method of identifying compounds capable of modulating TNF-α gene expression, which method comprises:

comparing the difference in the amount of reporter gene expression in the cells of claim 14 in the presence of the compound with the amount of reporter gene expression in the absence of the compound and/or with the amount of reporter gene expression in cells transfected with a control reporter gene expression construct which does not contain one or more of the sequences of nucleotides illustrated in SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, whereby compounds capable of modulating TNF-α gene expression are identified.

17. A method of identifying compounds capable of modulating TNF-α gene expression, which method comprises steps of:

(a) contacting an aqueous solution comprising a DNA binding protein as claimed in any one of claims 1 to 3 with a sample of the compound to form a reaction mixture;

(b) contacting the reaction mixture from part (a) with a DNA fragment comprising the sequence of nucleotides illustrated in SEQ ID NO: 1 or SEQ ID NO: 2; and

(c) observing the presence or absence of complexes comprising said DNA binding protein bound to said DNA fragment.

18. A method of identifying compounds capable of modulating TNF-α gene expression, which method comprises steps of:

(a) contacting an aqueous solution comprising the DNA binding protein claimed in any one of claims 1 to 3 and the transcription factor protein Oct-1 with a sample of the compound to form a reaction mixture ;

(b) contacting the reaction mixture of part (a) with a DNA fragment comprising the sequence of nucleotides illustrated in SEQ ID NO: 2; and

(c) observing the presence or absence of complexes comprising said DNA binding protein bound to said DNA fragment.

19. A method of identifying compounds capable of modulating TNF-α gene expression, which method comprises steps of:

contacting an aqueous solution comprising the DNA binding protein claimed in any one of claims 4 to 6 with a sample of the compound to form a reaction mixture;

(b) contacting the reaction mixture of part (a) with a DNA fragment comprising the sequence of nucleotides illustrated in SEQ ID NO: 3; and

(c) observing the presence or absence of complexes comprising said DNA binding protein bound to said DNA fragment.

20. A method as claimed in any one of claims 17 to 19 wherein said DNA fragment is radiolabelled and the presence or absence of complexes comprising said DNA binding protein bound to said DNA fragment is determined by electrophoretic mobility shift assay.

21. A method of identifying compounds capable of modulating TNF-α gene expression, which method comprises:

contacting a DNA-protein complex comprising one of the following DNA/protein combinations:

(i) a DNA fragment comprising the sequence of nucleotides illustrated in SEQ ID NO: 1 or SEQ ID NO: 2 and the DNA binding protein claimed in any one of claims 1 to 3,

(ii) a DNA fragment comprising the sequence of nucleotides illustrated in SEQ ID NO: 3 and the DNA binding protein claimed in any one of claims 4 to 6,

(ii) a DNA fragment comprising the sequence of nucleotides illustrated in SEQ ID NO: 2, the DNA binding protein claimed in any one of claims 1 to 3 and the transcription factor protein Oct-1,

with a sample of the compound and observing whether the DNA-protein complex is disrupted following contact with the compound.

22. A compound capable of modulating TNF-α gene expression, which compound has been identified using the method of any one of claims 15 to 21.

23. A nucleic acid molecule having the sequence of nucleotides illustrated in SEQ ID NO: 4.

24. A nucleic acid molecule as claimed in claim 23 which is a double-stranded DNA molecule.

25. A material comprising the nucleic acid molecule of claim 23 or claim 24 attached to a solid matrix or support.

26. A material as claimed in claim 25 wherein the nucleic acid molecule is labelled with biotin and

is attached to the solid support or matrix via a biotin/streptavidin binding interaction.

27. Use of the material claimed in claim 25 or claim 26 in a procedure for purifying a sequence-specific DNA binding protein as defined in any one of claims 1 to 3.

28. A nucleic acid molecule having the sequence of nucleotides illustrated in SEQ ID NO: 5.

29. A nucleic acid molecule as claimed in claim 28 which is a double-stranded DNA molecule.

30. A material comprising the nucleic acid molecule of claim 28 or claim 29 attached to a solid matrix or support.

31. A material as claimed in claim 30 wherein the nucleic acid molecule is labelled with biotin and is attached to the solid support or matrix via a biotin/streptavidin binding interaction.

32. Use of the material claimed in claim 30 or claim 31 in a procedure for purifying a sequence-specific DNA binding protein as defined in any one of claims 4 to 6.

33. A method of screening human individuals for predisposition to inflammatory disease, which method comprised screening for the presence of a G to A transition polymorphism at position -376 in the promoter region of the TNF-α gene.

34. A method as claimed in claim 33 wherein the inflammatory disease is cerebral malaria.

35. A method as claimed in claim 33 or claim 34 wherein said screening for the presence of a G to A transition polymorphism at position -376 in the promoter region of the TNF-α gene is carried out using PCR ELISA.

36. A method as claimed in claim 35 wherein said PCR ELISA is carried out using allele-specific oligonucleotide probes having the following sequences:

5'-CTGTCTGGAAGTTAGAAGGA (SEQ ID NO: 6)

5'-CTGTCTGGAAATTAGAAGGA (SEQ ID NO: 7)

SEQUENCE LISTING

<110> ISIS INNOVATION LIMITED

<120> MODULATOR OF INFLAMMATION

<130> SCB/50929/001

<140>
<141>

<160> 7

<170> PatentIn Ver. 2.0

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<223> Description of Artificial Sequence:
oligonucleotide probe for PCR ELISA

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PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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GRANDE BRETAGNE

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	30.10.2000
Applicant's or agent's file reference: SCB/50929/002	**REPLY DUE** **within 3 month(s)** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/00414	09/02/2000	09/02/1999

International Patent Classification (IPC) or both national classification and IPC

C12Q1/68

Applicant

ISIS INNOVATION LTD. et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☒	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☒	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain document cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4. For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis. For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 09/06/2001.

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Authorized officer / Examiner
Favre, N

Formalities officer (incl. extension f tim limits)
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Tel phone No. +49 89 2399 8161

I. Basis of the opinion

1. This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".*):

Description, pages:

1-34 as originally filed

Claims, No.:

1-36 as originally filed

Drawings, sheets:

1/7-7/7 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

see separate sheet

II. Priority

1. ☐ This opinion has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application,

☒ claims Nos. 22,

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 22 are so unclear that no meaningful opinion could be formed (*specify*):

see separate sheet

☒ the claims, or said claims Nos. 22 are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

IV. Lack of unity of invention

1. In response to the invitation (Form PCT/IPEA/405) to restrict or pay additional fees, the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with for th following reasons and chos , according to Rule 68.1, not to invite the applicant to restrict or pay additional fees:

see separat sheet

3. Consequently, the following parts of th international application were the subject of international preliminary

examination in establishing this opinion:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-3, 9-11, 23, 24, 33
Inventive step (IS)	Claims	4-8, 12-21, 28, 29, 34-36
Industrial applicability (IA)	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item I
Basis of the opinion

Sequence listing pages 1 and 2 filed on the 21.02.2000 have also been considered for the drafting of the present opinion (Rule 13ter.1(f) PCT).

Re Item II
Priority

The priority document does not disclose any material comprising immobilised nucleic acid molecules of SEQ ID NO: 4 and SEQ ID NO: 5, as claimed in claims 25-27 and 30-32 of the present application.
Therefore, the claimed priority is not valid for the subject-matter of claims 25-27 and 30-32.

Re Item III
Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claim 22 refers to a compound identified by the methods of claims 15-21. In the application as filed, there is however no example of any such compound (Rule 5.1 a)(v) PCT). Moreover, such a compound is not a product **directly produced** by the screening methods of claims 15-21, which relate to methods of identification. It is rather a product that has been **identified** using said methods.
Therefore, claim 22 is not considered to be supported by the description in the sense of Article 6 PCT.

Moreover, the subject-matter of claim 22 is also not considered to be sufficiently disclosed in the application as filed for the skilled person to carry them out and thus do

not meet the requirements of Article 5 PCT as there is no indication whatsoever in the application as filed of a compound falling within the scope of this claim.

According to the above objections, it is not possible to assess claim 22 for novelty and inventive step in the sense of Articles 33(2) and 33(3) PCT.

Re Item IV
Lack of unity of invention

The application as filed lacks unity within the meaning of Rule 13.1 PCT. However, it was chosen, according to Rule 68.1 PCT, not to invite the applicant to restrict or to pay additional fees.

The following groups of invention were found in the present application:

I) **Claims 1-3, 7-9, 12-16 (partially), 17,18, 20 (partially), 21 (partially), 23-27, and 33-36**: These claims define DNA binding proteins, nucleic acid molecules and methods, all of which relate to the α site (-404 - -374) of the *TNFα* promoter, said α site comprising a phenotypically relevant A to G transition polymorphism at position -376.

II) **Claims 4-6, 10, 11, 12-16 (partially), 19, 20 (partially), 21 (partially), and 28-32**: These claims define DNA binding proteins, nucleic acid molecules and methods, all of which relate to the β site (-371 - -352) of the *TNFα* promoter, said β site beginning 3 nucleotides downstream of the α site.

Apart from the fact that the α and β sites are immediately adjacent, there is no evidence, neither in the present application nor in the prior art, that both sites are functionally linked, for instance for the modulation of the TNFα gene expression. Therefore, the above two groups of inventions are not so linked by a special technical feature as to form a single general inventive concept (Rule 13.1 PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventiv step or industrial applicability; citations and explanations supporting such statement

1. Document D1 (Gene, 1993, **131**:307-308) discloses the nucleic acid sequence of the *TNFα* promoter region. In Figure 1, D1 discloses that the hepatocyte-specific nuclear protein-1 (H-APF-1) specifically binds to a DNA sequence corresponding to SEQ ID NO: 1.
   Moreover, it is known in the art, cf. document D2 (Nature Genetics, 1999, **22**:145-150) referring to a publication of Fletcher *et al.* in Cell, 1987, that OCT-1, another DNA binding protein, specifically binds to a DNA sequence corresponding to SEQ ID NO: 2.
   Therefore, the subject-matter of claim 1 is not novel in the sense of Article 33(2) PCT.

1.1 Dependent claims 2 and 3 further define the protein of claim 1 by a particular molecular weight (between 16 and 26 kDa) which is not a feature which is unique for only one protein, or by the use of a particular process for the isolation of said protein.
   Both features do not characterise the claimed proteins in such a way that it would differentiate said protein from the ones known from the prior art. Hence, the subject-matter of claims 2 and 3 is not novel in the sense of Article 33(2) PCT.

2. There is no indication in the prior art documents known to the International Preliminary Examination Authority of a protein specifically binding DNA having a sequence corresponding to SEQ ID NO: 3. The subject-matter of independent claim 4 is thus novel in the sense of Article 33(2) PCT.
   However, no technical problem appears to be solved by the protein defined in claim 4. Moreover, no unexpected or surprising technical effect appears to be associated with this protein, i.e. no modulation of the TNFα expression, or any other function, is shown to be related to the binding of this protein to SEQ ID NO: 3.

Therefore, the subject-matter of independent claim 4 and dependent claims 5 and 6, which further define said protein using non-characterising features (see also point 1.1 above), is not considered to be an invention, and thus is not considered to be inventive in the sense of Article 33(3) PCT.

3. Document D1 discloses the complete sequence of the *TNFα* promoter region (Fig. 1). In other words, the nucleic acid of D1 comprises the sequence illustrated in SEQ ID NO: 1.
The selection of a nucleic acid molecule restricted to the sequence of SEQ ID NO: 1 merely provides an alternative nucleic acid molecule which allow the recognition of a **known** polymorphic site (see also 3.1 below). Said selection is not be considered to be inventive in the sense of Article 33(3) PCT.

3.1 Document D3 (Journal of Inflammation, 1996, **46**:32-41) discloses that the -376 G to A transition polymorphism of the *TNFα* promoter region is known in the art. Therefore, modifying the sequence of D1 accordingly, thus resulting in a nucleic acid comprising the sequence illustrated in SEQ ID NO: 2, does not require an inventive activity from the person skilled in the art. Hence, independent claim 8 is not inventive in the sense of Article 33(3) PCT.

3.2 Claim 9 further define the nucleic acid molecules of claims 7 and 8 using intrinsic characteristics thereof. Therefore, the subject-matter of claim 9 is not novel in the sense of Article 33(2) PCT.

4. The complete sequence of the *TNFα* promoter region disclosed in D1 (Fig. 1) also comprises the sequence illustrated in SEQ ID NO: 3 and thus falls in the scope of claim 10. Therefore, independent claim 10 is not novel in the sense of Article 33(2) PCT.
Moreover, the selection of a nucleic acid molecule restricted to the sequence of SEQ ID NO: 3 would not be considered to be inventive as it is not associated with the solution of a technical problem and with any surprising effect.

4.1 For reasons similar to those put forward under point 3.2 above, claim 11 is also

not novel in the sense of Article 33(2) PCT.

5. Document D3, which is considered to represent the most relevant state of the art for the assessment of inventive step of claims 12-14, discloses (cf. page 34, column 1, line 3 - page 35, column 2, line 35) a reporter gene expression construct comprising the α and β sites of the *TNFα* promoter region from which the subject-matter of claim 12 differs in that nucleic acid sequences of the *TNFα* promoter region other than sequences corresponding to SEQ ID NO: 1, 2, or 3 have to be absent.

5.1 The problem to be solved by independent claim 12 may therefore be regarded as providing a reporter gene expression construct for the study of another polymorphic site of the *TNFα* promoter region.

5.2 As disclosed in D3 (page 33, paragraph bridging column 1 and 2) there are **only four** known G to A transition polymorphism. Therefore, choosing the polymorphism at position -376 is merely one of several straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill, in order to solve the problem posed.
Claim 12 and claim 13 which further define said construct with a feature which is standard for expression constructs are thus not inventive in the sense of Article 33(3) PCT.

5.3 Accordingly, a cell line as defined in claim 14 also does not fulfill the requirements of Article 33(3) PCT.

6. Document D3, which is also considered to represent the most relevant state of the art for the assessment of inventive step of claims 15-21, discloses (cf. page 34, column 1, line 3 - page 35, column 2, line 35) a method from which the subject-matter of claim 15 differs in that another reporter gene expression construct is used, i.e. a construct which does not comprise nucleic acid sequences of the *TNFα* promoter region other than sequences corresponding to SEQ ID NO: 1, 2,

or 3.

6.1 However, the reporter gene expression construct used in Example 5 of the present application (page 26, line 30) comprises the **complete** *TNFα* promoter region (~1.2 kb), i.e. comprises other factors which directly play a role in the modulation of the TNFα gene expression.
There is no indication whatsoever, either in the present application or in the prior art, that a reporter gene expression construct as claimed would lead to the identification of a compound capable of modulating TNFα gene expression.
Moreover, there is no indication whatsoever, either in the present application or in the prior art, that the nucleic acid sequence corresponding to SEQ ID NO: 3 plays any role in the modulation of the TNFα gene expression.

6.2 Modulation of the TNFα gene expression is the sole reason for the alleged inventiveness of these methods. The fact that the examples contained in the description show that the -376 polymorphism **in combination with** the rest of the **complete** *TNFα* promoter region plays a role in the modulation of the TNFα gene expression cannot not be regarded as sufficient evidence to lead to the inference that substantially all the claimed compounds possess this activity.
The requirements of Article 33(3) PCT are therefore not met by independent claim 15.

6.3 Similar objections also apply for the methods defined in claims 16-21 which thus do not meet the requirements of Article 33(3) PCT.

7. According to the objection of point 3. above, the nucleic acid molecule defined in claims 23 and 24 is not novel over D1 in the sense of Article 33(2) PCT.

7.1 According to the objection of point 4. above, the nucleic acid molecule defined in claims 28 and 29 is not inventive in the sense of Article 33(3) PCT.

8. Document D2, which is considered to represent the most relevant state of the art for the assessment of inventive step of claims 25-27 and 30-32 (see also Item II),

refers to the nucleic acid molecules of claims 23 and 28.
There is however no suggestion in D2 or in any prior art documents known to the International Preliminary Examination Authority that would prompt the skilled person to immobilise said nucleic acid molecules to a solid support, for example in order to enrich a sample in specific DNA binding proteins (page 31-33 of the description).
Thus, claims 25-27 and 30-32 are considered to be novel and inventive in the sense of Articles 33(2) and 33(3) PCT.

9. Document D4 (Molecular Medicine, 1998, **4**:724-733), discloses (cf. abstract) a method of screening human individuals for predisposition to an inflammatory disease, which method comprises screening for the presence of a G to A transition polymorphism at position -376 in the promoter region of the *TNFα* gene. The subject-matter of independent claim 33 is thus not novel in the sense of Article 33(2) PCT.

9.1 Thanks to the remarkable work of Dr. Kwiatkowski, the association between TNFα expression and cerebral malaria is well known in the art. Moreover, associations between G to A transition polymorphism in the *TNFα* promoter region and cerebral malaria are also known in the art, e.g. D3, page 40, column 1, lines 5-28. In addition, as disclosed in D3 (page 33, paragraph bridging column 1 and 2) there are **only four** G to A transition polymorphisms known in the *TNFα* promoter region. Therefore, using the method of D4 for screening human individuals for predisposition to cerebral malaria does not require an inventive activity from the skilled person. Claim 34 is thus not inventive in the sense of Article 33(3) PCT.

9.2 Finally, using PCR ELISA and oligonucleotide probes specific for each allele in a screening method require only routine selection from the person skilled in the art. Claims 35 and 36 are therefore not considered to be inventive in the sense of Article 33(3) PCT. Such a selection would however be considered to be inventive if associated with a surprising effect.

Re Item VIII
Certain observations on the international application

1. Although claims 15 and 16 have been drafted as separate independent claims, they appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is sought. The aforementioned claims therefore lack conciseness. Moreover, lack of clarity of the claims as a whole arises, since the plurality of independent claims makes it difficult to determine the matter for which protection is sought, and places an undue burden on others seeking to establish the extent of the protection. Hence, claims 15 and 16 do not meet the requirements of Article 6 PCT.

1.1 Similarly, independent claims 17-19 also appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is sought.
Claims 17-19 do thus also not meet the requirements of Article 6 PCT.

1.2 Furthermore, dependent claims 9 and 11 refer to intrinsic technical features and are thus redundant with independent claims 7 and 10, respectively. Claims 9 and 11 therefore lack conciseness in the sense of Article 6 PCT.

2. The embodiment of the invention described on pages 26-28 (Example 5) does not fall within the scope of the claims (see also Item V, points 6-6.3). This inconsistency between the claims and the description leads to doubt concerning the matter for which protection is sought, thereby rendering the claims unclear (Article 6 PCT).
The applicant is requested to remove the inconsistency, either by amending the claims, by deleting the "excess" subject-matter from the description and the drawings, or by indicating in the description that the embodiment concerned do not form part of the invention but represents background art useful for understanding the invention (see the PCT Guidelines, III-4.3).

2.1 Moreover, according to the above objection, claims 15-21 are not technically supported by the description in the sense of Article 6 PCT.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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GRANDE BRETAGNE

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PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	20.04.2001
Applicant's or agent's file reference: SCB/50929/002	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/00414	09/02/2000	09/02/1999

Applicant: ISIS INNOVATION LTD. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

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23 APR 2001
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Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
SCB/50929/002		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/00414	09/02/2000	09/02/1999
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant ISIS INNOVATION LTD. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 13 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 10 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08/09/2000	20.04.2001
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Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/00414

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-34 as originally filed

Claims, No.:

1-41 with telefax of 28/02/2001

Drawings, sheets:

1/7-7/7 as originally filed

Sequence listing part of the description, pages:

1-2, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequenc listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 22.

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 22 are so unclear that no meaningful opinion could be formed (*specify*):
   see separate sheet

   ☒ the claims, or said claims Nos. 22 are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-21, 23-41
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-3, 7-9, 23-27, 30-32, 36-41
	No:	Claims	4-6, 10-21, 28, 29, 33-35
Industrial applicability (IA)	Yes:	Claims	1-21 and 23-41
	No:	Claims	

2. Citations and explanations
s e separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
s s parate sh t

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claim 22 refers to a compound identified by the methods of claims 15-21. In the application as filed, there is however no example of any such compound (Rule 5.1 a)(v) PCT). Moreover, such a compound is not a product **directly produced** by the screening methods of claims 15-21, which relate to methods of identification. It is rather a product that has been **identified** using said methods.
Therefore, claim 22 is not considered to be supported by the description in the sense of Article 6 PCT.

Moreover, the subject-matter of claim 22 is also not considered to be sufficiently disclosed in the application as filed for the skilled person to carry them out and thus do not meet the requirements of Article 5 PCT as there is no indication whatsoever in the application as filed of a compound falling within the scope of this claim.

According to the above objections, it is not possible to assess claim 22 for novelty and inventive step in the sense of Articles 33(2) and 33(3) PCT.

Re Item IV

Lack of unity of invention

The application as filed lacks unity within the meaning of Rule 13.1 PCT. Howev r, it was chosen, according to Rule 68.1 PCT, not to invite the applicant to restrict or to pay additional fees.

The following groups of invention were found in the present application:

I) **Claims 1-3, 7-9, 12-16 (partially), 17,18, 20 (partially), 21 (partially), 23-27, 33-35, 36 (partially), 37, 38, 40 (partially) and 41**: These claims define DNA binding proteins, nucleic acid molecules and methods, all of which relate to the α site (-404 - -374) of the *TNFα* promoter, said α site comprising a phenotypically relevant A to G transition polymorphism at position -376.

II) **Claims 4-6, 10, 11, 12-16 (partially), 19, 20 (partially), 21 (partially), 28-32, 36 (partially), 39 and 40 (partially)**: These claims define DNA binding proteins, nucleic acid molecules and methods, all of which relate to the β site (-371 - -352) of the *TNFα* promoter, said β site beginning 3 nucleotides downstream of the α site.

Apart from the fact that the α and β sites are immediately adjacent, there is no evidence, neither in the present application nor in the prior art, that both sites are functionally linked, for instance for the modulation of the TNFα gene expression. Therefore, the above two groups of inventions are not so linked by a special technical feature as to form a single general inventive concept (Rule 13.1 PCT).

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step r industrial applicability; citations and explanations supporting such statement

1. Document D1 (Gene, 1993, **131**:307-308) discloses the nucleic acid sequence of the *TNFα* promoter region. In Figure 1, D1 discloses that the hepatocyte-specific nuclear protein-1 (H-APF-1) specifically binds to a DNA sequence corresponding to SEQ ID NO: 1.
Moreover, it is known in the art, cf. document D2 (Nature Genetics, 1999, **22**:145-150) referring to a publication of Fletcher *et al.* in Cell, 1987, that OCT-1, another DNA binding protein, specifically binds to a DNA sequence corresponding to SEQ ID NO: 2.
However, none of the prior art documents at hand discloses a DNA binding protein capable of binding specifically both to a DNA having the sequence of SEQ ID NO:1 and to a DNA having the sequence of SEQ ID NO:2. The subject-matt r of claim 1 is thus novel.
Furthermore, it is shown in the description that the binding of the claimed DNA binding protein is modulated by the known -376A/G mutation and by the presence of OCT-1 (page 9, lines 1-18) and it is shown that this mutation is associated with a modulation of the TNFα gene expression (e.g. Example 5). Hence, it is strongly suggested that the claimed DNA binding protein plays a role in the modulation of the TNFα gene expression. Since none of the prior art documents at hand fairly suggest that a DNA binding protein similar to that defined in claim 1 has a similar function, the subject-matter of claim 1 is also considered to be inventive.
Therefore, the subject-matter of independent claim 1 meets the requirements of Articles 33(2) and 33(3) PCT.

1.1 Dependent claims 2 and 3 further define the protein of claim 1, which is considered to be novel and inventive. Dependent claims 2 and 3 thus also meet the requirements of Articles 33(2) and 33(3) PCT.

2. There is no indication in the prior art documents known to the International Preliminary Examination Authority of a protein specifically binding DNA having a sequence corresponding to SEQ ID NO: 3. The subject-matter of independent

claim 4 is thus novel in the sense of Article 33(2) PCT.
However, the mere fact that a protein binds to a particular DNA, without any further technical effect, e.g. modulation of the TNFα expression, is not considered to be a solution to a technical problem. Hence, no technical problem appears to be solved by the protein defined in claim 4.
Therefore, the subject-matter of independent claim 4 and dependent claims 5 and 6, which further define said protein using non-characterising features (see also Item VIII), is not considered to be an invention, and thus is not considered to be inventive in the sense of Article 33(3) PCT.

3. Document D1 discloses the complete sequence of the *TNFα* promoter region (Fig. 1). In other words, the nucleic acid of D1 comprises the sequence illustrated in SEQ ID NO: 1.
Document D3 (Journal of Inflammation, 1996, **46**:32-41) discloses that the -376 G to A transition polymorphism of the *TNFα* promoter region is known in the art.
However, the selection of a nucleic acid molecules restricted to the sequence of SEQ ID NO: 1 or of SEQ ID NO: 2 provides nucleic acid molecules containing binding sites for the novel and inventive DNA binding protein of claim 1, and as such are useful for evaluating the binding of said protein.
Therefore, the subject-matter of claims 7-9 is considered to be a novel and inventive selection, and thus fulfils the requirements of Articles 33(2) and 33(3) PCT.

3.1 However, the complete sequence of the *TNFα* promoter region disclosed in D1 (Fig. 1) also comprises the sequence illustrated in SEQ ID NO: 3.
In the light of the argumentation of point 2. above, the selection of a nucleic acid molecule restricted to the sequence of SEQ ID NO: 3 cannot be considered to be inventive. Therefore, the subject-matter of claims 10 and 11 is not considered to meet the requirements of Article 33(3) PCT.

4. Document D3, which is considered to represent the most relevant state of the art for the assessment of inventive step of claims 12-14, discloses (cf. page 34, column 1, line 3 - page 35, column 2, line 35) a reporter gene expression

construct comprising the α and β sites of the *TNFα* promoter region from which the subject-matter of claim 12 differs in that nucleic acid sequences of the *TNFα* promoter region other than sequences corresponding to SEQ ID NO: 1, 2, or 3 have to be absent.

4.1 The problem to be solved by independent claim 12 may therefore be regarded as providing a reporter gene expression construct for the study of a polymorphic site of the *TNFα* promoter region recognised by a DNA binding protein.

4.2 In view of the argumentation of point 1. above, the solution of said technical problem comprising the selection of SEQ ID NO:1 and 2 would meet the requirements of the PCT. However, insofar that the claimed solution includes SEQ ID NO:3 and in view of the argumentation of point 2. above, the subject-matter of claim 12 and of claim 13, which further define said construct with a feature which is standard for expression constructs, is not considered to be inventive in the sense of Article 33(3) PCT.

4.3 Accordingly, a cell line as defined in claim 14 also does not fulfill the requirements of Article 33(3) PCT.

5. Document D3, which is also considered to represent the most relevant state of the art for the assessment of inventive step of claims 15-21, discloses (cf. page 34, column 1, line 3 - page 35, column 2, line 35) a method from which the subject-matter of claim 15 differs in that another reporter gene expression construct is used, i.e. a construct which does not comprise nucleic acid sequences of the *TNFα* promoter region other than sequences corresponding to SEQ ID NO: 1, 2, or 3.

5.1 However, the reporter gene expression construct used in Example 5 of the present application (page 26, line 30) comprises the **complete** *TNFα* promoter region (~1.2 kb), i.e. comprises other factors which directly play a role in the modulation of the TNFα gene expression.
There is no indication whatsoever, either in the present application or in the prior art, that a reporter gene expression construct as claimed would lead to the

identification of a compound capable of modulating TNFα gene expression. Moreover, there is no indication whatsoever, either in the present application or in the prior art, that the nucleic acid sequence corresponding to SEQ ID NO: 3 plays any role in the modulation of the TNFα gene expression.

5.2 Modulation of the TNFα gene expression is the sole reason for the alleged inventiveness of these methods. The fact that the examples contained in the description show that the -376 polymorphism **in combination with** the rest of the **complete** *TNFα* promoter region plays a role in the modulation of the TNFα gene expression cannot not be regarded as sufficient evidence to lead to the inference that substantially all the claimed compounds possess this activity.
The requirements of Article 33(3) PCT are therefore not met by independent claim 15.

5.3 Similar objections also apply for the methods defined in claims 16-21 which thus do not meet the requirements of Article 33(3) PCT.

6. According to the objection of point 3. above, the nucleic acid molecules defined in claims 23 and 24 are considered to be novel and inventive in the sense of Articles 33(2) and 33(3) PCT.

6.1 However, according to the objection of point 3.1 above, the nucleic acid molecules defined in claims 28 and 29 are not considered to be inventive in the sense of Article 33(3) PCT.

7. Document D2, which is considered to represent the most relevant state of the art for the assessment of inventive step of claims 25-27 and 30-32 (see also Item II), refers to the nucleic acid molecules of claims 23 and 28.
There is however no suggestion in D2 or in any prior art documents known to the International Preliminary Examination Authority that would prompt the skilled person to immobilise said nucleic acid molecules to a solid support, for example in order to enrich a sample in specific DNA binding proteins (page 31-33 of the description).

Thus, claims 25-27 and 30-32 are considered to be novel and inventive in the sense of Articles 33(2) and 33(3) PCT.

8. Document D4 (Molecular Medicine, 1998, **4**:724-733), discloses (cf. abstract) a method of screening human individuals for predisposition to an inflammatory disease, which method comprises screening for the presence of a G to A transition polymorphism at position -376 in the promoter region of the *TNFα* gen . The subject-matter of independent claim 34 differs from this disclosure in that it is specified that the inflammatory disease is cerebral malaria.
However, the association between TNFα expression and cerebral malaria is well known in the art. Moreover, associations between G to A transition polymorphism in the *TNFα* promoter region and cerebral malaria are also known in the art, e.g. D3, page 40, column 1, lines 5-28. Therefore, using the method of D4 for screening human individuals for predisposition to cerebral malaria does not require an inventive activity from the skilled person. Claim 33 is thus not inventiv in the sense of Article 33(3) PCT.

8.1 Finally, using PCR ELISA and oligonucleotide probes specific for each allel in a screening method require only routine selection from the person skilled in the art. Claims 34 and 35 are therefore not considered to be inventive in the sense of Article 33(3) PCT.

9. None of the prior art documents at hand discloses or fairly suggests methods of identifying compounds capable of disrupting a DNA/protein interaction occurring at the *TNFα* promoter, as defined in claims 36-41.
Said claims are thus considered to be novel and inventive in the sense of Articles 33(2) and 33(3) PCT.

Re Item VIII

Certain observations on the international application

1. Although claims 15 and 16 have been drafted as separate independent claims, they appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is sought. The aforementioned claims therefore lack conciseness. Moreover, lack of clarity of the claims as a whole arises, since the plurality of independent claims makes it difficult to determine the matter for which protection is sought, and places an undue burden on others seeking to establish the extent of the protection. Hence, claims 15 and 16 do not meet the requirements of Article 6 PCT.

1.1 Similarly, independent claims 17-19 also appear to relate effectively to the sam subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is sought.
Claims 17-19 do thus also not meet the requirements of Article 6 PCT.

1.2 A similar objection also applies for claims 37-39. Said claims do therefore not meet the requirements of Article 6 PCT.

2. The embodiment of the invention described on pages 26-28 (Example 5) does not fall within the scope of claims 12-21 (see also Item V, points 5-5.3). This inconsistency between the claims and the description leads to doubt concerning the matter for which protection is sought, thereby rendering the claims unclear (Article 6 PCT).

2.1 Moreover, according to the above objection, claims 12-21 are not experimentally supported by the description in the sense of Article 6 PCT.

3. Dependent claims 2 and 3 further define the protein of claim 1 by a particular molecular weight (between 16 and 26 kDa) which is not a feature which is unique for only one protein, or by the use of a particular process for the isolation of said protein. Both features do not characterise the claimed proteins, so that claims 2

and 3 are considered to be redundant and thus lack conciseness in the sense of Article 6 PCT.

3.1 A similar objection applies for claims 5 and 6, which thus do not meet the requirements of Article 6 PCT.

Claims:

1. A sequence-specific DNA binding protein which is capable of binding specifically both to a DNA molecule having the sequence of nucleotides illustrated in SEQ ID NO: 1 and to a DNA molecule having the sequence of nucleotides illustrated in SEQ ID NO: 2.

2. A sequence-specific DNA binding protein as claimed in claim 1 having an electrophoretic mobility equivalent to a protein of molecular weight 21 +/- 5kDa when run on an SDS PAGE denaturing gel.

3. A sequence-specific DNA binding protein as claimed in claim 1 or claim 2 which is obtainable by

(a) preparing a crude nuclear extract from the human monocyte cell line U937;

(b) applying the nuclear extract of part (a) to a column of heparin sepharose CL-6B or phosphocellulose P11;

(c) applying a salt gradient from 100 to 2000mM to the column;

(d) collecting a fraction of the eluate which is enriched for the said sequence-specific DNA binding protein;;

(e) incubating the enriched material obtained in step (d) with double-stranded DNA affinity probes comprising the following sequence:

5'-GTTCTATCTTTTTCCTGCATCCTGTCTGGAAGTTA
CAAGATAGAAAAAGGACGTAGGACAGACCTTCAAT-5'

to allow the formation of complexes of the sequence-specific DNA binding protein bound to the DNA affinity probe; and

(f) recovering the sequence-specific binding

protein from the complexes formed in step (e).

4. A sequence-specific DNA binding protein which is capable of binding specifically to a DNA molecule having the sequence of nucleotides illustrated in SEQ ID NO: 3.

5. A sequence-specific DNA binding protein as claimed in claim 4 having an electrophoretic mobility equivalent to a protein of molecular weight 30 +/- 5kDa when run on an SDS PAGE denaturing gel.

6. A sequence-specific DNA binding protein as claimed in claim 4 or claim 5 which is obtainable by:

(a) preparing a crude nuclear extract from the human monocyte cell line U937;

(b) applying the nuclear extract of part (a) to a column of heparin sepharose CL-6B or phosphocellulose P11;

(c) applying a salt gradient from 100 to 2000mM to the column;

(d) collecting the material eluted at 250-350mM salt for heparin sepharose or 500-600mM salt for P11 phosphocellulose;

(e) incubating the enriched material obtained in step (d) with double-stranded DNA affinity probes comprising the following sequence:

5'-TAGAAGGAAACAGACCACAGACCTG
ATCTTCCTTTGTCTGGTGTCTGGAC-5'

to allow the formation of complexes of the sequence-specific DNA binding protein bound to the DNA affinity probe; and

(f) recovering the sequence-specific binding protein from the complexes formed in step (e).

7. An isolated nucleic acid consisting of the sequence of nucleotides illustrated in SEQ ID NO: 1.

8. An isolated nucleic acid consisting of the sequence of nucleotides illustrated in SEQ ID NO: 2.

9. An isolated nucleic acid as claimed in claim 7 or claim 8 or a fragment thereof which is capable of specifically binding to the DNA binding protein of any one of claims 1 to 3.

10. An isolated nucleic acid consisting of the sequence of nucleotides illustrated in SEQ ID NO: 3.

11. An isolated nucleic acid as claimed in claim 10 or a fragment thereof which is capable of specifically binding to the DNA binding protein of any one of claims 4 to 6.

12. A reporter gene expression construct comprising:

a reporter gene encoding a transcriptional and/or translational product which can be directly or indirectly detected; and

a transcriptional control element comprising one or more of the sequences of nucleotides illustrated in SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 in the substantial absence of any other nucleotide sequence from the TNF-α promoter, the transcriptional control element being operably linked to the reporter gene.

13. A reporter gene expression construct as claimed in claim 12 which further comprises one or more *cis*-acting promoter or enhancer elements from a heterologous promoter.

14. Cells transformed or transfected with th

reporter gene expression construct of claim 12 or claim 13.

15. A method of identifying a compound capable of modulating TNF-α gene expression, which method comprises:

contacting a fragment of DNA comprising one or more of the sequences of nucleotides illustrated in SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 in the substantial absence of any other nucleotide sequences from a TNF-α promoter with a sample of the compound and detecting any specific binding of the compound to the fragment of DNA.

16. A method of identifying compounds capable of modulating TNF-α gene expression, which method comprises:

comparing the difference in the amount of reporter gene expression in the cells of claim 14 in the presence of the compound with the amount of reporter gene expression in the absence of the compound and/or with the amount of reporter gene expression in cells transfected with a control reporter gene expression construct which does not contain one or more of the sequences of nucleotides illustrated in SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, whereby compounds capable of modulating TNF-α gene expression are identified.

17. A method of identifying compounds capable of modulating TNF-α gene expression, which method comprises steps of:

(a) contacting an aqueous solution comprising a DNA binding protein as claimed in any one of claims 1 to 3 with a sample of the compound to form a reaction mixture;

(b) contacting the reaction mixture from part

(a) with a DNA fragment comprising the sequence of nucleotides illustrated in SEQ ID NO: 1 or SEQ ID NO: 2; and

(c) observing the presence or absence of complexes comprising said DNA binding protein bound to said DNA fragment.

18. A method of identifying compounds capable of modulating TNF-α gene expression, which method comprises steps of:

(a) contacting an aqueous solution comprising the DNA binding protein claimed in any one of claims 1 to 3 and the transcription factor protein Oct-1 with a sample of the compound to form a reaction mixture ;

(b) contacting the reaction mixture of part (a) with a DNA fragment comprising the sequence of nucleotides illustrated in SEQ ID NO: 2; and

(c) observing the presence or absence of complexes comprising said DNA binding protein bound to said DNA fragment.

19. A method of identifying compounds capable of modulating TNF-α gene expression, which method comprises steps of:

contacting an aqueous solution comprising the DNA binding protein claimed in any one of claims 4 to 6 with a sample of the compound to form a reaction mixture;

(b) contacting the reaction mixture of part (a) with a DNA fragment comprising the sequence of nucleotides illustrated in SEQ ID NO: 3; and

(c) observing the presence or absence of complexes comprising said DNA binding protein bound to said DNA fragment.

20. A method as claimed in any one of claims 17 to 19 wherein said DNA fragment is radiolabelled and

the presence or absence of complexes comprising said DNA binding protein bound to said DNA fragment is determined by electrophoretic mobility shift assay.

21. A method of identifying compounds capable of modulating TNF-α gene expression, which method comprises:

contacting a DNA-protein complex comprising one of the following DNA/protein combinations:

(i) a DNA fragment comprising the sequence of nucleotides illustrated in SEQ ID NO: 1 or SEQ ID NO: 2 and the DNA binding protein claimed in any one of claims 1 to 3,

(ii) a DNA fragment comprising the sequence of nucleotides illustrated in SEQ ID NO: 3 and the DNA binding protein claimed in any one of claims 4 to 6,

(ii) a DNA fragment comprising the sequence of nucleotides illustrated in SEQ ID NO: 2, the DNA binding protein claimed in any one of claims 1 to 3 and the transcription factor protein Oct-1,

with a sample of the compound and observing whether the DNA-protein complex is disrupted following contact with the compound.

22. A compound capable of modulating TNF-α gene expression, which compound has been identified using the method of any one of claims 15 to 21.

23. A nucleic acid molecule consisting of the sequence of nucleotides illustrated in SEQ ID NO: 4.

24. A nucleic acid molecule as claimed in claim 23 which is a double-stranded DNA molecule.

25. A material comprising the nucleic acid molecule of claim 23 or claim 24 attached to a solid

matrix or support.

26. A material as claimed in claim 25 wherein the nucleic acid molecule is labelled with biotin and is attached to the solid support or matrix via a biotin/streptavidin binding interaction.

27. Use of the material claimed in claim 25 or claim 26 in a procedure for purifying a sequence-specific DNA binding protein as defined in any one of claims 1 to 3.

28. A nucleic acid molecule consisting of the sequence of nucleotides illustrated in SEQ ID NO: 5.

29. A nucleic acid molecule as claimed in claim 28 which is a double-stranded DNA molecule.

30. A material comprising the nucleic acid molecule of claim 28 or claim 29 attached to a solid matrix or support.

31. A material as claimed in claim 30 wherein the nucleic acid molecule is labelled with biotin and is attached to the solid support or matrix via a biotin/streptavidin binding interaction.

32. Use of the material claimed in claim 30 or claim 31 in a procedure for purifying a sequence-specific DNA binding protein as defined in any one of claims 4 to 6.

33. A method of screening human individuals for predisposition to cerebral malaria, which method comprised screening for the presence of a G to A transition polymorphism at position -376 in the promoter region of the TNF-α gene.

34. A method as claimed in claim 33 wherein said screening for the presence of a G to A transition polymorphism at position -376 in the promoter region of the TNF-α gene is carried out using PCR ELISA.

35. A method as claimed in claim 34 wherein said PCR ELISA is carried out using allele-specific oligonucleotide probes having the following sequences:

5'-CTGTCTGGAAGTTAGAAGGA (SEQ ID NO: 6)

5'-CTGTCTGGAAATTAGAAGGA (SEQ ID NO: 7)

36. A method of identifying a compound capable of disrupting a DNA/protein interaction occurring at the TNFα promoter, which method comprises:

contacting a fragment of DNA comprising one or more of the sequences of nucleotides illustrated in SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 in the substantial absence of any other nucleotide sequences from a TNF-α promoter with a sample of the compound and detecting any specific binding of the compound to the fragment of DNA.

37. A method of identifying compounds capable of disrupting a DNA/protein interaction occurring at the TNFα promoter, which method comprises steps of:

(a) contacting an aqueous solution comprising a DNA binding protein as claimed in any one of claims 1 to 3 with a sample of the compound to form a reaction mixture;

(b) contacting the reaction mixture from part (a) with a DNA fragment comprising the sequence of nucleotides illustrated in SEQ ID NO: 1 or SEQ ID NO: 2; and

(c) observing the presence or absence of complexes comprising said DNA binding protein bound to said DNA fragment.

38. A method of identifying compounds capable of disrupting a DNA/protein interaction occurring at the TNFα promoter, which method comprises steps of:

(a) contacting an aqueous solution comprising the DNA binding protein claimed in any one of claims 1 to 3 and the transcription factor protein Oct-1 with a sample of the compound to form a reaction mixture ;

(b) contacting the reaction mixture of part (a) with a DNA fragment comprising the sequence of nucleotides illustrated in SEQ ID NO: 2; and

(c) observing the presence or absence of complexes comprising said DNA binding protein bound to said DNA fragment.

39. A method of identifying compounds capable of disrupting a DNA/protein interaction occurring at the TNFα promoter, which method comprises steps of:

contacting an aqueous solution comprising the DNA binding protein claimed in any one of claims 4 to 6 with a sample of the compound to form a reaction mixture;

(b) contacting the reaction mixture of part (a) with a DNA fragment comprising the sequence of nucleotides illustrated in SEQ ID NO: 3; and

(c) observing the presence or absence of complexes comprising said DNA binding protein bound to said DNA fragment.

40. A method as claimed in any one of claims 37 to 39 wherein said DNA fragment is radiolabelled and the presence or absence of complexes comprising said DNA binding protein bound to said DNA fragment is determined by electrophoretic mobility shift assay.

41. A method of identifying a compound capable of disrupting a DNA/protein interaction occurring at the TNFα promoter, which method compris s:

contacting a DNA-protein complex comprising one of the following DNA/protein combinations:

(i) a DNA fragment comprising the sequence of nucleotides illustrated in SEQ ID NO: 1 or SEQ ID NO: 2 and the DNA binding protein claimed in any one of claims 1 to 3,

(ii) a DNA fragment comprising the sequence of nucleotides illustrated in SEQ ID NO: 3 and the DNA binding protein claimed in any one of claims 4 to 6,

(ii) a DNA fragment comprising the sequence of nucleotides illustrated in SEQ ID NO: 2, the DNA binding protein claimed in any one of claims 1 to 3 and the transcription factor protein Oct-1,

with a sample of the compound and observing whether the DNA-protein complex is disrupted following contact with the compound.

EUROPEAN PATENT OFFICE,
Erhardtstrasse 27,
D-80331 MUNICH,
GERMANY.

27 February 2001

Dear Sirs,

International Patent Application No. PCT/GB00/00414
ISIS INNOVATION LIMITED et al.
Representative's Ref: SCB/50929/002

I write in response to the Written Opinion dated 30 October 2000 issued in respect of the above-referenced International Patent Application and enclosed herewith amended claims 1-41 to replace the claims presently on file.

Amendments under Art. 34 PCT

In the amended claims submitted herewith, claim 1 has been amended to recite that the sequence-specific DNA binding protein is capable of binding specifically to a DNA molecule having the sequence of nucleotides illustrated in SEQ ID NO: 1 AND to a DNA molecule having the sequence of nucleotides illustrated in SEQ ID NO: 2. Basis for this amendment is to be found in the application as filed. In particular, the EMSA experiment of Example 3 clearly demonstrates that complex 1 is formed with both α_G and α_A probes, confirming that the 21 kDa protein identified by the inventors is capable of specifically binding to both SEQ ID NO: 1 and SEQ ID NO: 2.

Independent claims 7, 8, 10, 23 and 28 have been amended such that they relate to nucleic acids consisting of the sequences of SEQ ID Nos 1, 2, 3, 4 and 5, respectively, in the absence of any contiguous sequences from the TNFα promoter. The sequences illustrated as SEQ ID Nos 1-5 are shown in the specification as fragments isolated from the remainder of the TNFα promoter, in Figures 1, 2, 4 and 7. It is therefore clearly contemplated to use these sequences in isolation from any contiguous sequences from the TNFα promoter.

Independent claim 33 has been amended to relate to a method of screening human individuals for predisposition to cerebral malaria. Basis for the amendment is to be found in former dependent claim 34.

New claims 36 to 41 are directed to a method of identifying a compound which is capable of disrupting a DNA/protein interaction occurring at the TNFα promoter. Basis for the amended claims is to be found in original claims 15 and 17- 21 and on page 9, at lines 19-27, where it is clearly stated that disruption of the novel DNA-protein binding interactions at the TNFα promoter forms the basis of the assays designed to identify compounds capable of modulating TNFα expression.

Priority

The Examiner's attention is drawn to the passage on page 5, at lines 4-15, of the priority document which clearly describes the use of synthetic oligonucleotides comprising sequences corresponding to site α or site β of the TNFα promoter attached to a solid support such as, for example, a column matrix.

Novelty-claim 1

The Examiner is of the opinion that the subject-matter of claim 1 lacks novelty in view of document D1. D1 is concerned with sequencing of a 1.2kb fragment of the human TNFα promoter region. Several potential binding sites for DNA binding proteins were identified in this sequence by computer-assisted comparison of the TNFα sequence to published sequences of DNA binding sites. Although a potential binding site for the protein H-APF-1 was identified on this basis there is no evidence in D1 to show that H-APF-1 actually can bind to a DNA molecule corresponding to SEQ ID NO:1. In contrast, the present inventors have identified a protein which is capable of specifically binding to such a molecule on the basis of DNA footprinting and electrophoretic mobility shift assay.

In the amended claims filed herewith claim 1 has been amended to recite that the sequence-specific DNA binding protein is capable of binding specifically to a DNA molecule having the sequence of nucleotides illustrated in SEQ ID NO: 1 AND to a DNA molecule having the sequence of nucleotides illustrated in SEQ ID NO: 2. There is no disclosure or even suggestion in D1 that H-APF-1 might be capable of binding to a DNA molecule corresponding to SEQ ID NO: 2. Furthermore, the work of the present inventors has demonstrated that the single nucleotide substitution at position -376 of the TNFα promoter has a profound effect on the recruitment of DNA binding proteins to the α site. Thus, even if D1 did show that H-APF-1 is actually capable of specifically binding to a DNA molecule corresponding to SEQ ID NO: 1 there is no basis in the disclosure of D1 for concluding that H-APF-1 is also capable of binding to a DNA molecule corresponding to SEQ ID NO: 2. Accordingly, it is submitted that claim 1, and claims 2 and 3 which are dependent thereon, are novel over document D1.

The Examiner further considers claim 1 to lack novelty over the protein OCT-1. The basis for this objection is document D2 which, in the opinion of the Examiner, discloses OCT-1 to be capable of specifically binding to a DNA sequenc which corresponds to SEQ ID NO: 2. In the amended claims filed herewith claim 1 has been amended to recite that the sequence-specific DNA binding protein is

capable of binding specifically to a DNA molecule having the sequence of nucleotides illustrated in SEQ ID NO: 1 AND to a DNA molecule having the sequence of nucleotides illustrated in SEQ ID NO: 2. The experimental data included in the present application clearly shows that the protein OCT-1 is NOT capable of binding to both DNA sequences corresponding to both SEQ ID No: 1 and to DNA sequences corresponding to SEQ ID NO: 2. The EMSA study in particular shows that complex II comprising both OCT-1 and the 21 kDa protein forms only at the α site of the TNF_{-376A} allele and not the TNF_{-376G} allele (see Figure 5). Accordingly, on the basis of its binding specificity the sequence-specific DNA binding protein of claim 1 is novel over OCT-1. Claims 2 and 3 are also novel by dependency from claim 1. In addition, claim 2 contains the further feature that the protein has an electrophoretic mobility equivalent to a protein of molecular weight 21 +/- 5 kDa when run on an SDS PAGE denaturing gel. This feature alone distinguishes the claimed protein from OCT-1, which has a molecular weight of ~95 kDa.

Claim 1 is also inventive since there is nothing in the prior art which suggests the existence of a sequence-specific DNA binding protein capable of binding to both the sequence illustrated as SEQ ID NO: 1 and the sequence illustrated as SEQ ID NO: 2. The finding of a protein which has binding specificity for both sequences cannot be considered obvious.

Inventive step-claim 4

The Examiner acknowledges that claim 4 is novel, since there is no disclosure in the prior art of a sequence-specific DNA protein capable of specifically binding to a DNA molecule corresponding to SEQ ID NO: 3. However, claim 4 is considered to lack inventive step because, in the opinion of the Examiner, no technical problem is solved by the protein of claim 4 and no unexpected or surprising effect is associated with this protein. It is submitted that in the absence of any teaching or suggestion in the prior art that the sequence corresponding to SEQ ID NO: 3 contains a binding site for a sequence-specific DNA binding protein, the finding of a protein which binds specifically to this sequence cannot be considered obvious. The finding of a 30 kDa protein binds to this sequence is unexpected in view of the prior art, since there is nothing in the prior art to suggest the existence of a protein which functions as a sequence-specific DNA binding protein capable of binding to this particular region of the TNFα promoter. Accordingly, claim 4 and dependent claims 5 and 6 are all inventive in view of the cited prior art.

Novelty and inventive step-claims 7-11, 23-24 and 28-29

In the amended claims submitted herewith, claims 7 and 8 have been amended to more clearly relate to nucleic acid molecules consisting of the sequences illustrated in SEQ ID NO: 1 and SEQ ID NO: 2, respectively, in the absence of contiguous sequences from the TNFα promoter. Both claims are novel having regard to the disclosure in D1 of the complete sequence of the TNFα promoter region. The selection of the sequences illustrated as SEQ ID NO:1 and SEQ ID NO:2 from the remainder of the

TNFα promoter provides isolated nucleic acid molecules containing binding sites for the novel 21 kDa DNA binding protein identified by the present inventors. These nucleic acid molecules are useful for evaluating the binding of the 21 kDa protein to its target site in the TNFα promoter and represent an inventive selection from the TNFα promoter region as a whole.

Claim 10 has similarly been amended to more clearly relate to a nucleic acid molecule consisting of the sequence illustrated in SEQ ID NO: 3 in the absence of contiguous sequences from the TNFα promoter. This sequence is also novel having regard to the complete sequence of the TNFα promoter region disclosed in D1. The selection of the sequence illustrated as SEQ ID NO: 3 from the remainder of the TNFα promoter provides an isolated nucleic acid molecule containing the binding site for the novel 30 kDa DNA binding protein identified by the present inventors. This nucleic acid molecule is useful for evaluating the binding of the 30 kDa protein to its target site in the TNFα promoter and represents an inventive selection from the TNFα promoter region as a whole.

Similar considerations apply to the nucleic acid sequences of amended claims 23 and 28. In each case, the claimed nucleic acid represents a novel selection from the TNFα promoter sequence as a whole. Furthermore, the claimed nucleic acids are useful as affinity probes for the purification of the 21 kDa and 30 kDa proteins, respectively.

Inventive step-claims 12-14

Independent claim 12 is considered to lack inventive step in view of document D3. Document D3 discloses a reporter gene expression construct comprising a -619/+108 fragment of TNFα upstream of a reporter gene. The reporter gene expression construct of claim 12 is novel over D3, since it contains sequences corresponding to SEQ ID NO: 1, 2 or 3 in the absence of other sequences from the TNFα promoter. The sequences illustrated as SEQ ID NO: 1, 2 and 3 have been shown by the present inventors to contain specific binding sites for DNA binding proteins. Furthermore, the inventors have shown that the -376 G/A polymorphism occurring within this region has a direct effect on transcription factor recruitment. Since there is no disclosure in the prior art that these sequences actually contain specific binding sites for DNA binding proteins it would not be obvious to one of ordinary skill in the art to select these particular regions from TNFα promoter. The significance of these regions as providing binding sites for specific DNA binding proteins only becomes apparent in the light of the studies carrying out by the present inventors.

The Examiner is of the opinion that the problem to be solved by claim 12 is to be regarded as providing a reporter gene expression construct for the study of another polymorphic site of the TNFα promoter region and that choosing the polymorphism at position -376 is merely one of several straightforward possibilities from which the skilled person would select in order to solve this problem. It is submitted that in formulating the problem to be solved in these terms the Examiner has not taken account of the significance of the sequences of SEQ ID NO: 1, 2 and 3 as providing binding sites for specific DNA binding proteins. It is further suggested that based on the teaching of D3 the obvious solution to the

problem posed by the examiner would be simply to use constructs analogous to those shown in Figure 1 of D3 containing the 'wild type' G at position -308 and either G or the polymorphic A at position -376. There is no teaching in D3, or indeed the remainder of the prior art cited by the Examiner, which would provide any incentive to select just the sequences of SEQ ID NO: 1, 2 and 3 for inclusion into a construct if the sole aim is merely analysis of the -376 polymorphism. Accordingly, claim 12 and also claims 13 and 14 are all inventive over document D3.

Novelty and inventive step-claim 33

In the amended claims filed herewith, claim 33 has been amended to refer to a method of screening human individuals for predisposition to cerebral malaria and is therefore novel over document D4.

The Examiner is of the opinion that use of the method of D4 for screening human individuals for predisposition to cerebral malaria does not require inventive activity because (i) associations between G to A transition polymorphisms in the TNFα promoter region and cerebral malaria are known from document D3 and (ii) there are only four G to A transition polymorphisms known in the TNFα promoter region. In paragraph 5.2 the Examiner suggests that choosing the polymorphism at -376 is merely one of several straightforward possibilities from which the skilled person would select without the exercise of inventive skill. In fact, the selection of the -376 polymorphism is based on the novel biological findings of the present inventors, particularly the DNA footprinting data which shows that the -376 polymorphism occurs within a site of protein-DNA interaction, the EMSA data which shows the effect of the -376 polymorphism on transcription factor recruitment and the reporter gene expression data. Taken together, this data provides strong evidence for the functional significance of the -376 polymorphism. These findings led the present inventors to select the -376 polymorphism as being a likely marker for cerebral malaria. The association between the -376 polymorphism and cerebral malaria was then clearly demonstrated by a population-based study. In summary, the -376 polymorphism was selected by the inventors on the basis of experimental evidence regarding the functional significance of this polymorphism in the regulation of TNFα expression. This selection cannot be considered obvious on the basis of the cited prior art.

New claims 36-41

New claims 36-41 relate to methods of identifying compounds capable of disrupting DNA/protein interaction(s) occurring at the TNFα promoter. The present inventors have clearly demonstrated the occurrence of novel protein-DNA interactions at the TNFα promoter on the basis of DNA footprinting and EMSA analysis. The methods of the invention are selective for compounds which disrupt these interactions and may therefore be useful as modulators of TNFα expression.

Favourable re-consideration of this application is respectfully requested in the light of the amended claims filed herewith and the comments set out above.

Please acknowledge safe receipt of this letter by stamping and returning the enclosed EPO Form 1037.

Yours faithfully,

BALDOCK; Sharon Claire
Authorised Representative

: 269601: NLW: NLW: LONDOCS